

## **ImageStream® IgD Membrane Capping**

### **Membrane Immunoglobulin Capping on Murine B Lymphocytes**

#### **Experimental Procedures**

##### **Samples:**

1) Single fluorescent color control samples – start with  $3 \times 10^6$  total cells each. In this experiment, controls are: unlabeled

IgD/rat anti-mouse biotin/SA-Alexa Fluor 488  
CD19 PE

At the end, combine and resuspend in 75  $\mu$ l 1%PFA

2) Experimental samples – start with  $3 \times 10^6$  total cells. Stain according to following protocol. At the end, resuspend in 75  $\mu$ l 1%PFA.

Unless indicated, all stains and washes performed with Staining Buffer (SB, 1%FCS in PBS)

#### **Materials**

01. rat anti mouse IgD (clone 11-26c.2a) 1:100 (BD Cat.# 553438,0.5mg/ml) or other primary antibody
  02. PharM Lyse (10X, ammonium chloride lysing reagent) : BD Cat.# 555894)
  03. Fetal Calf Serum
  04. Phosphate Buffered Saline
  05. Staining Buffer (SB) = 1% FCS in PBS
  06. Staining Buffer with azide (SBaz) = 1% FCS, 0.9% azide in PBS (BD)
  07. FITC mouse anti-rat IgG, Fc $\gamma$  specific 1:100 (1.6 mg/ml: Jackson ImmunoResearch Cat#212-095-104)
  08. PE anti-mouse CD19 1:10 (100 ug/ml Caltag)
- Note : BD (BD Biosciences , BD PharMingen)

#### **Animals**

Female BALB/c mice, 9-11 weeks of age

#### **Staining of surface IgD**

01. Prepare spleen single cell suspension in 1x PharM Lyse to deplete RBC.
02. Filter through 40 micron mesh and top with complete RPMI.
03. Count the cell number
04. Spin Cells, aspirate supernatant

## **IgD Membrane Capping**

07. Resuspend  $2 \times 10^7$  cells/ml in SB (capping) or SBaz (non-capping) + anti mouse IgD ( $5\mu\text{g/ml}/2 \times 10^7$  cells), 10'  $4^\circ\text{C}$
08. Add 10X volume SB to the tube, and mix gently.
09. Spin cells at  $4^\circ\text{C}$ , aspirate supernatant.

### **Capping (or non-capping) Studies**

01. Resuspend at  $2 \times 10^7$  cells/ml SB plus 1:100 dilution of FITC-mouse anti-rat IgG,  $\text{Fc}\gamma$  15 minutes at room temp (for capping) or in SBaz for 5' @ RT (for non-capping).
02. wash, resuspend  $2 \times 10^7$  cells/ml SBaz plus 1:10 dilution of PE anti-mouse CD19 for 5 minutes at  $4^\circ\text{C}$
03. wash 2x SBaz, resuspend  $3 \times 10^7$  cells/ml in 1%PFA, and run on the IS100